Dynamics of Fecal Coliform and Culturable Heterotroph Densities in an Eutrophic Ecosystem: Stability of Models and Evolution of These Bacterial Groups

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Abstract. Time series of a population of fecal coliforms and a community of total viable counts were recorded during years 5 and 6 after the "birth" of an eutrophic aquatic ecosystem (sewage treatment lagoons). These time series were used to re-examine models, previously published, describing their temporal dynamics as well as the relationships between bacterial and environmental variables. The dynamics of the fecal coliforms and their relationships to the environment were unchanged; the fecal coliform abundances displayed an annual cycle with maximum reduction in numbers during the summer, which would be due at least partly to environmental variables (hypotheses of control by irradiance and pH, which have a seasonal behavior, are supported by the data). On the contrary, the total viable count dynamics moved towards a closer dependence on phytoplankton, from a situation of relative independence with respect to other biotic components of the ecosystem. Indeed during the first two years, only one of the abiotic variables in the model (the biological oxygen demand, which is an indicator of available organic matter) seemed to have an effect on the total viable counts. The behavior of these bacterial groups, measured during 1980–1982 and 1984–1986, shows that demographic and ecological laws founded on the observation of other organisms also apply to heterotrophic bacteria. A population, such as the fecal coliforms in the present study, has a limited ecological amplitude and is then more likely to react to environmental variables such as irradiance, pH, and phytoplanktonic metabolic products, whose bactericidal action is highest during the summer months and lowest during winter. On the other hand, a community such as that detected by the total viable counts of the present study is composed of many species and thus has a larger ecological amplitude. This makes it easier for the species to occupy the various available habitats and to maintain themselves through ecological succession and endogenous rhythms.

Introduction

In previous papers, [7, 15] we described the spatio-temporal dynamics of pollution indicator bacteria and of total viable counts, as well as the way these

bacteria are controlled by environmental variables in an aquatic eutrophic ecosystem; the ecosystem under study consists of the lagoons of an urban wastewater center. The dynamic and the explanatory models proposed in these papers are based upon a sampling program that started at the time of "birth" of the system (when sewage first arrived in the lagoon) and was pursued for 26 months (sampling at two-week intervals between June 1980 and August 1982). In the present paper, we report on the evolution of the bacterial compartment after 2 years to investigate the stability or the modifications of their dynamic behavior and of their relationships with limnological variables. These new results will also allow us to validate one of the models reported by Troussellier et al. [15] using independent data.

Materials and Methods

Our study was carried out at the sewage treatment center of the city of Mèze (03°35′06″E, 43°25′10″N) in southern France, on the shore of the Thau brackish water basin. This system was previously described by Baleux and Troussellier [1] and by Legendre et al. [7]. In the present study, only one station was examined. Located at the exit of the sewage treatment center, this station was chosen because, among the eight that had been sampled in previous studies, it displayed the clearest temporal evolution for fecal coliform counts (FC) and for total viable counts (TVC), leading to the statistical demonstration of different temporal behaviors for these two bacterial groups. It was also for the fecal coliform counts recorded at this station that we had obtained the highest coefficient of determination in our models [15]. On the other hand, total viable counts showed a larger numerical independence with respect to environmental cyclic variables at this same station.

  This station was sampled every 4 weeks between October 1984 and September 1986, consistently around 10 a.m. The sampling procedure and the counting method (spread-plate) have been described by Legendre et al. [7].

  Environmental variables, recorded concurrently with the bacterial abundances, were limited to those that had been found previously to be the most significant in explaining the temporal dynamics of bacteria : pH, irradiance (variable E, in $W \cdot cm^{-2}$) and chlorophyll a concentration (variable Chl a, in $\mu g \cdot liter^{-1}$). Why these variables are the most important to explain bacterial evolution and how they are recorded were reported by Troussellier et al. [15].

  The statistical methods used to analyze these new data series were basically the same as in the first 2-year study: (a) chronological clustering [8] and contingency periodogram [6], to search respectively for succession steps and for periodic phenomena in time series; (b) multiple or simple linear regressions (Model I and Model II) to verify the initial models or to elaborate new ones.

  To check if the new data fit the FC model's predictions, we used only that part (3 factors) of the 1980–1982 regression model that was found to have a significant direct influence on the fecal coliforms at the exit of the treatment plant. The effect of these factors is expressed by the following equation (where z designates standardized variables, i.e., transformed by subtracting the mean and dividing by the standard deviation):

$$zFC = +0.514zFC_B - 0.388zpH - 0.156zE \tag{1}$$

where FC and FC_B are the fecal coliform concentrations ($\log_e (x + 1) \cdot ml^{-1}$) at time t and $(t - 1)$ respectively, pH is the pH value at time t and E is the $\log_e$ of the irradiance value for the ten-day period prior to sampling. During the 1984–1986 sampling period, the mean value was 7.78 (range: 6.8–8.5) for the pH variable, and 0.017 $W \cdot cm^{-2}$ (range: 0.006–0.032) for irradiance, E. This last variable was used instead of the R variable (transmitted radiant energy) used in the initial model, because the water transparency data needed for the estimation of R were not available for the 1984–1986 survey. Variable E shows a strong natural periodicity. Variable pH presents a higher-frequency variability, but the lowest and highest values appear respectively in winter and in spring or autumn periods. All variables included in the regressions were tested and found not to deviate
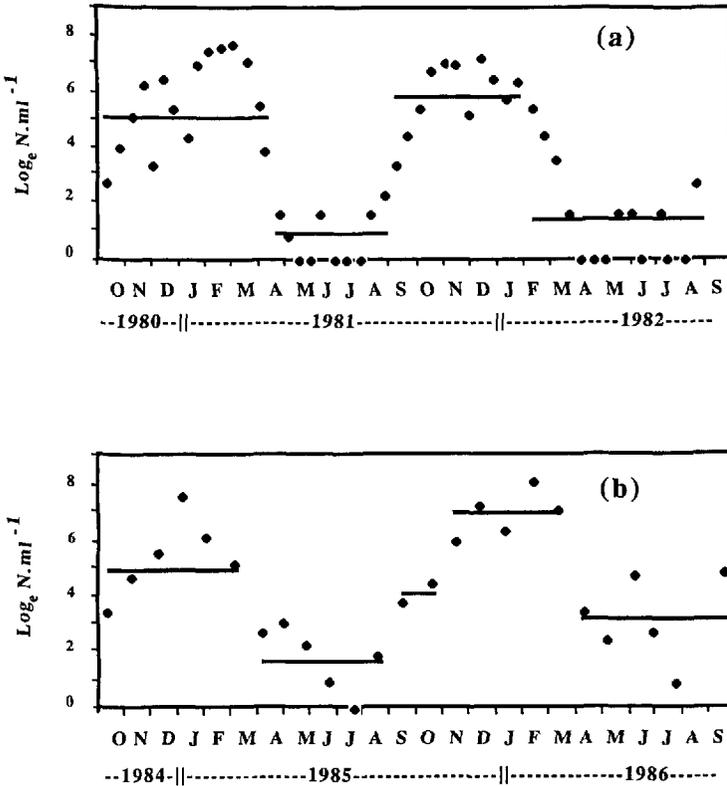
Fig. 1. Temporal evolution of fecal coliforms (FC, in $\log_e$ values) during the first (a) and the second (b) surveys. Horizontal line segments represent the partition of each series by chronological clustering ($\alpha = 0.05$).

significantly from normality, according to a Kolmogorov-Smirnov test of goodness-of-fit [9]. We have verified the adequacy of this "limited" equation to fit the values of FC observed in 1980–1982: $R^2 = 0.867$ instead of $R^2 = 0.881$ obtained with the complete equation given by Troussellier et al. [15]. In that paper, the equation, written in standardized form (z in equation 1, designating standardized variables), was used to initially compare the effects of several independent variables on the same dependent variable. In order to use it for prediction, Eq. 1 needs to be transformed to a conventional, unstandardized form [14]. The new but equivalent equation becomes:

$$FC = 36.548 + 0.529FC_B - 3.419pH - 0.789E \qquad (2)$$

Predicted values of FC for 1984–1986 were obtained by using in Eq. 2 the observed values of the three independent variables, as measured during the 1984–1986 survey.

A new regression model will be proposed for the 1984–1986 TVC data, for reasons given below.

Results

Evolution of the Dynamics of Bacterial Abundances

Figure 1 represents the temporal evolution of the fecal coliform counts at the exit of the treatment plant, from the birth of the ecosystem (June 1980) to 2
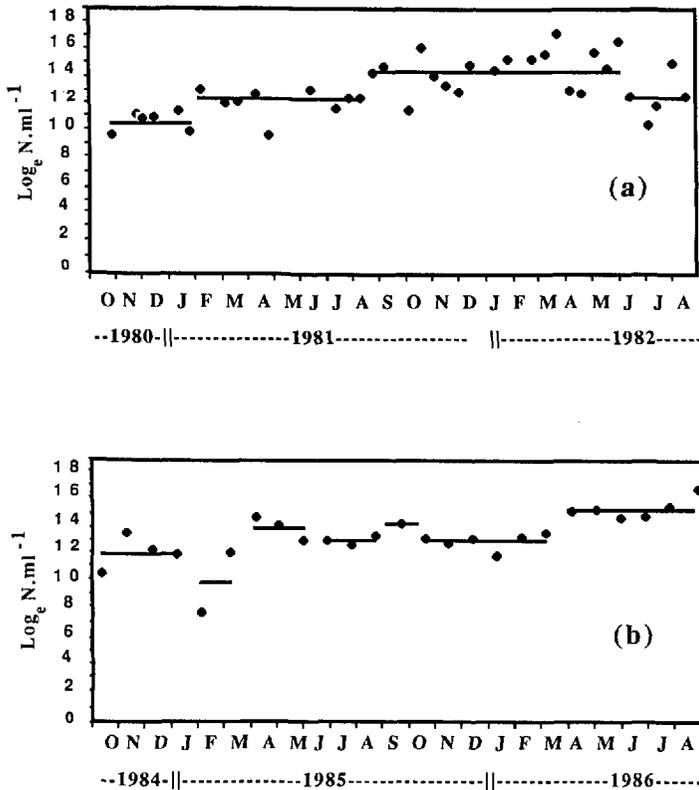
Fig. 2. Temporal evolution of the total viable counts (TVC, in log$_e$ values) during the first (a) and the second (b) surveys. Horizontal line segments represent the partition of each series by chronological clustering ($\alpha = 0.10$).

years later (August 1982) (Fig. 1a) and from October 1984 to September 1986 (Fig. 1b). The results of chronological clustering are also presented: each chronological step is characterized by the mean of the log$_e$-transformed data points contained in it.

The two series display very little differences. In the "new" as well as in the "old" series, there is a succession of significantly different high and low bacterial abundances, occurring respectively in winter and in summer time. A contingency periodogram analysis confirmed that four years after the "birth" of the system, fecal coliform counts still show a significant annual periodicity (for periods $T = 12$ or 13 intervals of 4 weeks, $P < 0.005$) in the output of the treatment plant while this periodicity is not significant in the incoming wastewater where the FC variable is relatively stable (coefficient of variation = 0.037, for the log$_e$ transformed data), as was the case for the 1980–1982 series [7]. Our previous study [15] has supported the hypothesis that this cyclic component in 1980–1982 was due to the seasonal cycle of the controlling environmental variables; the present paper will show that the situation is still the same in the 1984–1986 data series.

Chronological clustering results for the total viable counts variable are presented in Figure 2. To illustrate the evolution of the bacterial behavior, we
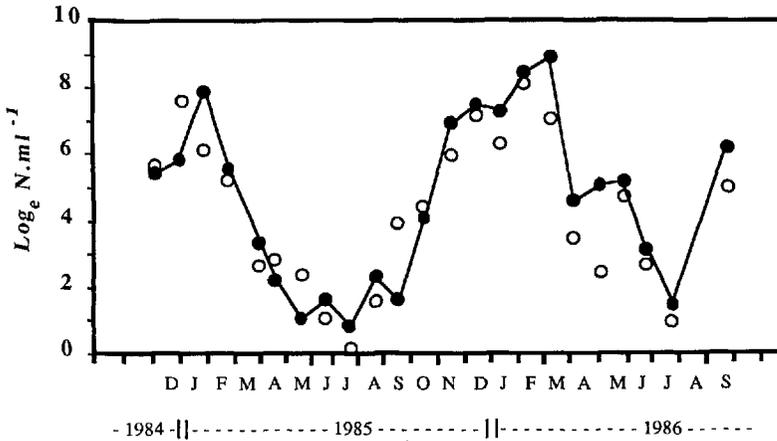
Fig. 3. Temporal evolution of the fecal coliforms (FC, in $\log_e$ values). Observed values (open circles); values predicted by the model (closed circles).

report on separate graphs the evolution of the two successive TVC series. From October 1984 to September 1986 (Fig. 2b), TVC are relatively stable compared to the FC results (Fig. 1b). This stability is also different from the marked stepwise increase of TVC observed during the initial survey (1980–1982; Fig. 2a).

No significant periodicity was detected in the TVC data and the range of fluctuation is relatively small (from 10^5 to 10^7 cells·ml^{-1}). This supports our previous hypothesis [15] that the FC on the one hand and the TVC on the other hand are controlled by different processes.

Evolution of the Relationships Between Bacterial and Limnological Variables

The likeness between the two time series of fecal coliform counts suggests that the predictive model (Eq. 2) built from the data obtained during the first survey (1980–1982) may be suitable to predict the dynamics of fecal coliforms two years later.

Figure 3 shows the observed and the predicted values of fecal coliform concentrations. The mean and 95% confidence limit values of the parameters of the Model II regression (reduced major axis) fitted between the observed and the predicted FC data are the following:

slope = 0.9312   95% C.I. = [0.7383, 1.1241]
intercept = −0.0126   95% C.I. = [−0.9081, 0.8828]

Reduced major axis was used here because D. J. Currie, P. Legendre, and A. Vaudor (submitted for publication) have shown that this is the most appropriate technique to express a functional relation when the slope is expected to be close to 1. This is the case here since we hypothesize a perfect prediction of the observed values by the model, i.e., a regression equation with a slope of one and an intercept of zero. The 95% confidence intervals reported above

show this to be the case. This conclusion is also supported by the highly significant percentage of variance of the FC variable explained by the model ($R^2 = 0.78$, $P \le 0.0001$) which confirms its ability to predict the evolution of fecal coliforms up to 6 years after the first sewage input. It also serves to validate the model, since the new 1984–1986 data are independent from those (1980–1982) used to compute the model's parameters.

The 1980–1982 model was far less successful at predicting TVC abundances. We had interpreted the monotonic trend of gradual increase in TVC during the first 2 years of life of this ecosystem as a colonization strategy of the heterotrophic niche, supported by a continuous increase in available organic matter (as estimated by the biological oxygen demand variable). The colonization hypothesis was supported by the numerical independence of the bacterial community from the cyclic environmental variables [15]; the environmental variables tested in the model were chlorophyll *a*, rotifers, solar radiation, pH, % saturation in dissolved oxygen, and temperature. The existence of a new behavior in 1984–1986 (Fig. 2b) for these bacteria, i.e., global temporal stability with a few peaks, suggests that some variables of the ecosystem, rather than external cyclic variables, could affect TVC. One such variable, whose effect on environmental bacteria is well documented in aquatic ecosystems, is phytoplankton. To verify the hypothesis that TVC peaks are related to phytoplankton blooms, we used simple linear regression which leads to the best fit of the dependent variable's values, in the least squares sense, even under Model II.

For the 1984–1986 series, a significant relationship can be found, not between phytoplankton (whose biomass is estimated by Chl *a*) and bacterial concentrations (TVC) measured at the same time *t* (linear regression not significant), but between the Chl *a* data ($\log_e$-transformed) at time $(t - 1)$, i.e., 4 weeks before, and the TVC ($\log_e$-transformed) at time *t*. This linear equation is:

$$TVC = 1.94 \text{Chl } a(t - 1) + 2.395 \qquad (3)$$

In this case the value of the coefficient of determination is highly significant ($R^2 = 0.73$; $P \le 0.0001$) and higher than the one obtained with a different model for the data from the 1980–1982 survey ($R^2 = 0.48$) [15].

Discussion

From a practical point of view, the FC results indicate first that the dynamic of the bacterial purification performance of this wastewater treatment system is relatively stable through time. The seasonal cycle is maintained 6 years after the first wastewater input (minima in summer and maxima in winter). The initial explanatory and predictive model is also corroborated, i.e., new FC data fit the model's predictions. So, the fecal coliforms remain dependent upon environmental variables that have a cyclic seasonal behavior.

On the contrary, if one compares the temporal dynamics of TVC during the first 2 years of survey (1980–1982) with the one observed 2 years later (1984–1986), we find two different and successive strategies of demographic development and niche occupancy for the heterotrophic bacterial community: (a) a slow but regular strategy of gradual occupation of the heterotrophic niche,

followed by (b) a stationary behavior with variations that are controlled by endogenous processes of the ecosystem. The first strategy is supported, at least partly, by the organic matter unused upstream (as measured previously by the biological oxygen demand variable) but appears to be independent of the seasonal fluctuations of the limnological variables [15]. While the behavior that follows in later years is also independent of cyclic seasonal rhythms, it begins to follow positively the fluctuations of one of the living components of the sewage lagoon ecosystem (phytoplankton biomass estimated by Chl *a*), instead of the unused organic matter loading that has become quite stable after the years of maturation of the system. This is demonstrated by the very close statistical relationship between TVC and Chl *a* discussed above. This positive relation is observed only if the model includes a time lapse, as in the classical predatory-prey models [10, 16]: the maxima of Chl *a* concentration take place before those of the heterotrophic bacteria, as can be expected of any organism whose abundance depends upon a fluctuating resource. Indeed, the positive effect of Chl *a* on TVC is likely to be the consequence of either an increasing excretion of dissolved organic matter by senescent cells of phytoplankton or of the decomposition of dead algal cells. This kind of influence is extensively described in the literature and has been experimentally demonstrated [2, 3, 12].

As mentioned in an earlier paper [15], sewage treatment lagoons are of fundamental interest for bacterial ecology, not only for their sanitary implication, but also from the viewpoint of theoretical ecology. They make it possible to study in short space and time spans the behavior of natural ecosystems and the interactions among their living components.

The survey of two different types of bacterial variables (a population, i.e., FC, and a community, i.e., TVC), through the successive steps of evolution (birth, maturity) of the lagoon, gives us the opportunity to verify in situ that bacteria do not "escape" the theoretical rules of ecology and demography, which have been founded on the observation of other groups of living beings. The population of FC shows a limited ecological capacity for interaction, being initially adapted to a very close and stable niche (the digestive tract). When it is transplanted into an open environment, it is likely to react to the bactericidal action of environmental variables such as irradiance, pH, and phytoplankton metabolism products. These bactericidal actions are highest during the summer months and lowest during winter, so that the demographic pattern of the FC becomes simple (cyclic) and repetitive when they are submitted to the lagoon environmental conditions. Such limited population behavior has already been noted for other kinds of bacterial populations in aquatic ecosystems [1, 5]. As an entity, the heterotrophic bacterial community presents on the contrary, by definition, a larger ecological spectrum and shows a more diversified demographic pattern of responses to the evolution of their environment, so that it is easier for them to occupy the various habitats available and to maintain themselves through ecological succession. In this way, the community of viable heterotrophic bacteria seems to be a good indicator of the evolution of an ecosystem moving away from an oligotrophic state and towards eutrophicity. The lagoons were indeed oligotrophic before June 1980 (initiation of sewage input in the first lagoon). Up to then, they had been flooded with rain water,

and it took 7 months before the biological oxygen demand variable reached the value of 50 mg·liter^{-1} in the third lagoon.

The change in the dynamics of TVC between the two surveys indicates that instead of having a short life span, this lagoon wastewater treatment system reaches an ecological equilibrium, providing an illustration of the establishment of endogenous rhythms as can be found in ecosystems reaching maturity [4, 11]. This ecological rule is rooted in a fundamental theorem in mathematical thermodynamics by Morowitz [13], stating that energy and/or matter exchanges imply the emergence of cycles. The maturation of an ecosystem increases the complexity of these exchanges, leading to the creation of cycles with different time spans. The periods of the cycles of the various interacting variables interfere with one another, giving rise to new cycles. Since these cycles result from the intrinsic behavior of the ecosystem, there is no reason for them to be adjusted to exogenous rhythms (the seasonal cycle, for example). So, we can see how an ecosystem reaching maturity can produce its own rhythms and fluctuations, whose importance grows with the development of internal interactions, such as between components of the heterotrophic bacterial community and components of the phytoplankton community. Detailed measurement and dynamic modelling of these components would be necessary to fully validate this mechanism and show how it can generate the fluctuations actually observed in the bacterial communities of mature ecosystems.

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